# The Influence of Dominance Rank on the Reproductive Success of Female Chimpanzees

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*Science* **277**, 828 (1997);
DOI: 10.1126/science.277.5327.828



Science (print ISSN 0036-8075; online ISSN 1095-9203) is published weekly, except the last week in December, by the American Association for the Advancement of Science, 1200 New York Avenue NW, Washington, DC 20005. 

domains are indicators of cell fate. The fates of the cells arising from different mitotic domains had been based on existing fate maps that were generated by visual inspection of unmarked living or fixed embryos. These predictions were confirmed for the domains analyzed thus far. However, direct marking of these cells revealed much more detail about the actual array of fates.

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3. GAL4VP16 was caged according to the following protocol. To one volume of a 1.35 mg/ml solution of the protein in storage buffer (as determined with the Bradford assay) was first added one volume of a 0.1 M sodium carbonate buffer (pH 9.7) and then two volumes of NVOC-Cl (Fluka) in 1,4-dioxane. The pH of the buffer was such that the final pH of the reaction was 9.5. The reaction was carried out for 30 min at room temperature and was stopped by the addition of one volume of acidic 0.1 M tris-HCl, which also lowered the pH to 7.4. GAL4VP16 was found to be very stable under the reaction conditions used. The caged protein was washed twice with Centricon C-30 devices (Amicon) in a 20 mM Na-Hepes, 0.2 M NaCl solution (pH 7.3). The unirradiated, caged GAL4VP16 was stable at −80°C for more than 12 months.
4. The extent of caging was determined by fluorescamine labeling of free amines as described [P. Böhlen, S. Stein, W. Dairman, S. Udenfried, *Arch. Biochem. Biophys.* **155**, 213 (1973)]. Parallel samples of ~30 μg of GAL4VP16 were treated with or without NVOC-Cl according to the standard caging protocol but were washed instead with 50 mM sodium phosphate buffer (pH 8). The samples were then brought to a final volume of 1.5 ml containing 1% SDS and were boiled for 3 min. Fluorescamine (0.5 ml, 30 mg/100 ml; Molecular Probes) in 1,4-dioxane was added, and the fluorescence of the sample was analyzed with a fluorimeter (excitation at 390 nm, emission at 485 nm). Results of the caged protein samples were correlated with that of the control sample that was incubated without NVOC-Cl.
5. Embryos were prepared for injection as described [J. S. Minden, D. A. Agard, J. W. Sedat, B. M. Alberts, *J. Cell Biol.* **109**, 505 (1989)]. All injections were done during interphase 14. For immunostaining, embryos were fixed and manually devitellinized as described [J. P. Vincent and J. P. O'Farrell, *Cell* **68**, 923 (1992)]. After devitellinization, embryos were washed briefly in methanol (30 s), and antibody staining was carried out by standard protocols as reported [H. M. Bomze and A. J. Lopez, *Genetics* **136**, 965 (1994)]. 5C.2B (anti-UBX) was diluted 1:65, 4CI-I (anti-ANTP) was diluted 1:33, anti-GAL4 (Santa Cruz Biotechnology) was diluted 1:1000, and anti–β-Gal (Sigma) was diluted 1:1500.
6. Presumably, this level of modification inhibited GAL4VP16 binding to chromatin, rather than naked DNA, or interfered with specific protein-protein interactions. Anti-GAL4 staining showed that caging did not perturb nuclear localization (*12*).
7. Ultraviolet doses for photoactivation were quantitated by actinometry as described [C. G. Hatchard and C. A. Parker, *Proc. R. Soc. London.* **A235**, 518 (1956)] with an assumed quantum yield of 1.26 at 366 nm. *Drosophila* photoactivations were done with a UPlanFl 60×/1.25 oil-immersion objective on an Olympus IX-70 microscope with an Olympus IX-RFA/Caged attachment. The beam used for photoactivation of single mitotic cells in *Drosophila* embryos delivered an energy dose of about 8 μJ per second. Thus, a total of 24 μJ were necessary for photoactivation of a single cell. Photoactivation of entire *Xenopus* embryos required about 82-fold as much energy per area than individual *Drosophila* cells. In vitro photoactivation with the hand-held UV lamp was less efficient than in the in vivo experiments by a factor of about 8200, which explains the long irradiation times necessary for the gel mobility-shift assay.
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14. A 400-μm pinhole was used for single-cell irradiation in the amnioserosa tissue and a 600-μm pinhole was used for single mitotic cells.
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17. Mitotic domains were identified by differential interference contrast microscopy of mitotic cells, which swell up and protrude above the nondividing epithelium. Alternatively, mitotic domains were visualized by fluorescence confocal microscopy of a transgenic strain that constitutively expressed a nuclear-localized GFP (*23*). The different mitotic domains were identified by the time of appearance, the location, and the shape of the mitotic patch. ∂2 is a golf club–shaped group of cells that form immediately anterior to the cephalic furrow, with the head of the golf club at the ventral midline and the shaft oriented parallel to the cephalic furrow. ∂8 forms a circular cluster of cells that straddle the ventral midline anterior to ∂2. ∂8 is completely surrounded by ∂15, which forms a single row of cells separating ∂8 from ∂2, ∂9, ∂1, and ∂23. Stained tissues were identified on the basis of their morphology, location, and embryonic origin (*24*).
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19. Albino *Xenopus* embryos were injected with 5 nl into each blastomere at the 4-cell stage with 0.05 mg/ml caged or unmodified GAL4VP16 and 0.0125 mg/ml $UAS_G GFP$ (S65T) plasmid (GFP was fused to a nuclear localization signal). Embryos were kept at 19° to 20°C at all times and were injected superficially into the animal hemisphere. At 19°C, embryos reached stage 9 in 12 hours and stage 31/32 in about 2 days. Embryos were irradiated for 45 to 60 s with UV light from a standard UV filter set (Chroma Technology) on a Zeiss Axiophot, with the use of a 10×/0.5 Fluar air-lens.
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26. The fluorescence intensity units plotted are corrected for background fluorescence. For each time point, the fluorescence value at $t = 0$ of the respective curve was subtracted. The original uncorrected fluorescence units at $t = 0$ were 975 (□), 949 (●), 857 (○), and 1078 (▲).


27. We thank V. Hartenstein for help with the identification of the stained tissues and J. Ambroziak for help with the gel mobility-shift assays. The $UAS_G lacZ$ stock was provided by N. Perriman. The $UAS_G Ubxla$ stock and anti-UBX antibody was provided by J. Lopez. We thank B. Schmidt and members of the reagent group at the Science and Technology Center for Light Microscope Imaging and Biotechnology for helpful comments and W. McClure, J. Lopez, D. L. Taylor, A. Koretsky, and members of the Minden lab for critical review of the manuscript. Supported by a NSF Training Grant Fellowship, a Lucille Markey Charitable Trust Interdisciplinary Program in Biotechnology Fellowship, and the Friedrich-Naumann-Stiftung (S.B.C.) and by the Medical Foundation (Fleet Bank Trustee) (R.L.D.) and NSF Center for Light Microscope Imaging and Biotechnology grant BIR-8920118 (J.S.M.). J.S.M. is a Lucille Markey Scholar. This work was supported in part by a grant from the Lucille P. Markey Charitable Trust.

6 March 1997; accepted 27 June 1997




# The Influence of Dominance Rank on the Reproductive Success of Female Chimpanzees

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Female chimpanzees often forage alone and do not display obvious linear dominance hierarchies; consequently, it has been suggested that dominance is not of great importance to them. However, with the use of data from a 35-year field study of chimpanzees, high-ranking females were shown to have significantly higher infant survival, faster maturing daughters, and more rapid production of young. Given the foraging behavior of chimpanzees, high rank probably influences reproductive success by helping females establish and maintain access to good foraging areas rather than by sparing them stress from aggression.

In many species of group-living mammals, especially those that feed on monopolizable foods, such as spotted hyenas and many primates, females have frequent dominance interactions and are ranked in stable linear hierarchies (*1–4*). These hierarchies result from, and are maintained by, a pattern of alliances in which close relatives support each other against more distant relatives and high-ranking matrilines support each

other against low-ranking matrilines (*5*). In most studies, high rank is associated with higher reproductive success (*3*, *6*), although this relation is often weak, perhaps because of counterbalancing costs of high rank (*7*). There is debate over the extent to which the effects of rank on reproductive success are due to better access to food for high-ranking individuals or to protection from stress that results from aggression toward individuals at the bottom of the hierarchy (*7*, *8*).

Chimpanzees resemble these species in that they live in permanent social groups and feed predominantly on ripe fruit that often occurs in monopolizable patches (*9*). However, they differ in that females in some populations spend more than half their time feeding alone, and most females disperse to other groups before breeding, with the result that they are usually not surrounded by relatives (*9*). Compared to female macaques and baboons, it is difficult to detect linear hierarchies among female chimpanzees. Although some female chimpanzees clearly dominate others (*10*–*13*), dominance behavior in stable groups or stable pairs of females is uncommon and is never observed between some dyads (*10*, *13*). In addition, when aggressive behavior does occur within a dyad, it is sometimes two-sided with no clear winner (*14*). These observations have led some to believe that female dominance is unimportant for reproductive success (*13*, *15*). However, others have suggested that dominant females may gain advantages (*10*, *16*). Here we present data from a 35-year study of the chimpanzees of Gombe National Park, Tanzania.

The chimpanzees of Gombe have been studied since 1960 (*10*). The 48.7-km$^2$ park consists of a series of steep valleys running from the eastern rift escarpment (1600 m elevation) to Lake Tanganyika (775 m). The valley bottoms contain evergreen forest that gives way to semi-deciduous forest on the valley sides and grassland on the ridges (*17*). Since 1963, the chimpanzees of the central area of the park have been provisioned with bananas at an artificial feeding station in order to habituate them and to facilitate regular observation (*10*). The feeding station has been likened to an unusually long-term natural food source and, since 1970, has been estimated to provide less than 2% of the chimpanzees' diet (*18*, *19*). Daily observations are made of the presence, reproductive state, and social interactions of individuals at the feeding station and, since 1975, during daily all-day follows of individuals throughout their range (*10*). Since 1970, the habituated community has consisted of 4 to 13 adult males, 10 to 18 adult females, and 18 to 31 immatures and has occupied a range of 6.75 to 14.5 km$^2$ spanning three to six main valleys in the middle of the park (*10*, *20*). Adult females spend about 65% of their time alone with only their dependent offspring, foraging in distinct but overlapping core areas of about 2 km$^2$ (*19*, *21*), whereas adult males are more social, travel over the whole community range, and jointly patrol and defend its borders (*10*, *19*). Whereas almost all males born in the community remain in the community as adults, most or all natal females visit other communities during adolescence, and about 50% emigrate permanently (*22*).

We assessed dominance relationships among females by examining the direction of all pant-grunts between females recorded from 1970 to 1992. Pant-grunts are the most reliable measure of submission in chimpanzees and correlate with the reception of aggressive behavior (*13*, *14*). When we constructed dyadic matrices, many cells were empty, but by assessing 2-year blocks we were able to assign 88% of the females that were observed more than 10 days per year (*23*) as high-, middle-, or low-ranking in each block (*24*). Dominance rank was not related to body weight ($R^2$ = 0.01, $n$ = 15) (*25*, *26*), but individual dominance rank increased with age (*27*) as observed in the chimpanzees of the Mahale Mountains (*11*). However, a female's rank at age 21 strongly predicts her rank a decade later ($R^2$ = 0.80, $P$ = 0.001, $n$ = 9), suggesting that early rank acquisition is important.

Dominance rank has a marked effect on several measures of reproductive success. First, offspring survival was significantly related to mother's rank at the birth of her offspring. Infants of low-ranking females showed much higher mortality than those of high-ranking females over the first 7 years of life (Fig. 1). Second, the age at which daughters reached sexual maturity was significantly related to their mother's dominance rank (Fig. 2). Daughters of low-ranking females experienced their first full anogenital swelling during which adult males mated with them (*28*) as much as 4 years later than daughters of high-ranking females. Age at first full swelling was strongly correlated with age at first birth ($R^2$ = 0.67, $P$ = 0.01, $n$ = 8 regularly observed females). Third, there was a tendency for high-ranking females to live longer (*29*). Finally, the annual production of offspring surviving to weaning age (5 years) was correlated with rank for mature females that were observed for at least 12 years (Fig. 3), indicating that low-ranking females were unable to compensate for the higher mortality of their offspring by reproducing more quickly. All these factors combined to produce higher lifetime reproductive success in females of higher rank (*30*). In the analyses concerning reproduction, we excluded the female GG, because she was sterile. GG was an aggressive, masculine-

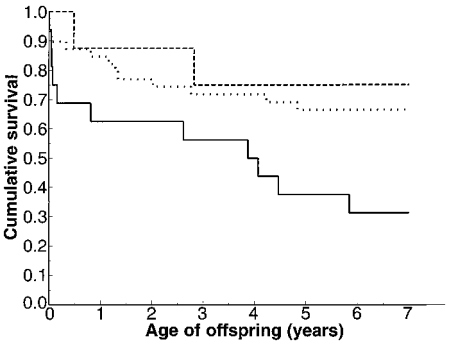


**Fig. 1.** Kaplan-Meier cumulative survival plot of offspring at ages 0 to 7 for females of high, middle, and low rank at the birth of the offspring. Dashed line indicates high rank ($n$ = 10 infants of 4 mothers), dotted line, middle rank ($n$ = 39 infants of 13 mothers), and solid line, low rank ($n$ = 16 infants of 11 mothers). In a single Cox-proportional hazards regression with multiple variables (*38*), mother's rank and age at birth were both significant factors. The exponentiated coefficients estimate the proportional increase in infant mortality rates as a function of unit change in rank, such as from middle to low, or increase in age; Exp(coeff.$_{rank}$) = 4.3, $P$ = 0.001, Exp(coeff.$_{age}$) = 1.09, $P$ = 0.01.

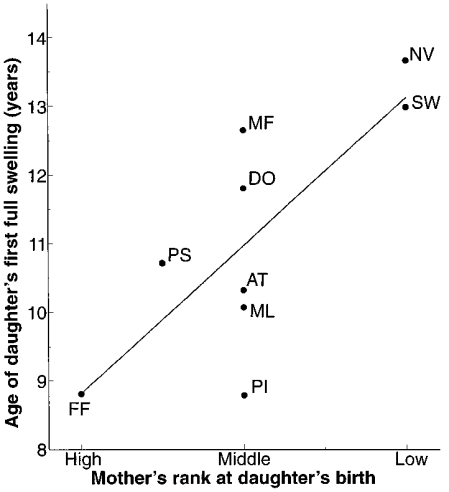


**Fig. 2.** Age of daughter's reproductive maturity plotted against mother's rank at the birth of her daughter ($R^2$ = 0.59, $P$ = 0.02, $n$ = 9 females). For the one mother with two mature daughters, we used the mean age of maturity. Mother's average rank over the daughter's lifetime was also a significant indicator of daughter's age of maturity ($R^2$ = 0.58, $P$ = 0.02).

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looking female who occupied the highest rank and cycled regularly for 28 years but never became pregnant. If she is included in the analyses, the relation of the rate of production of surviving infants and lifetime reproductive success with rank ceases to be significant (Fig. 3) (*30*).

Dominance probably exerts its effects in several ways. First, the high mortality of infants of low-ranking females in the first few months of life was partly due to the infanticidal behavior of the high-ranking female, PS, and her daughter, PM, who snatched and ate the infants of several females in the 1970s (*10*, *31*). Since then, high-ranking females FF and GG were observed trying to snatch the newborn infant of middle-ranking female GM, and females of unknown rank in an adjacent community were seen eating another female's infant (*20*). These observations suggest that female infanticide may be a significant, if sporadic, threat, rather than the pathological behavior of one female. However, infants are vulnerable to infanticide for only a few weeks, and rank-related effects on offspring mortality continue well beyond that age (Fig. 1). Second, the younger age at which daughters of high-ranking females reach sexual maturity reflects their higher rates of weight gain (*20*), suggesting that high-ranking females have better nutrition. Better nutrition might also account for better survival of high-ranking females and their offspring. In species living in permanent groups, reduced reproductive success of low-ranking females has been attributed to chronic stress due to frequent aggression from other females (*8*). Because female chimpanzees spend so much time alone, often going for a day or longer without seeing another female, this is unlikely to be important in this species.

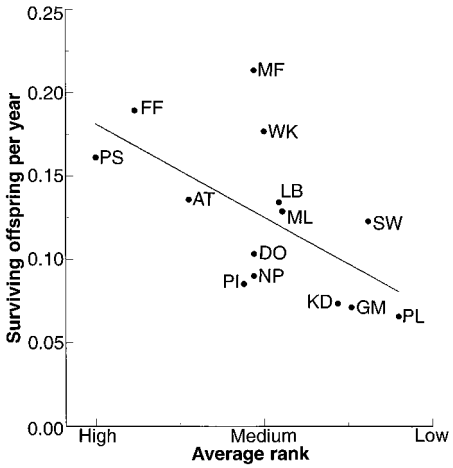


**Fig. 3.** Annual production of infants surviving to age 5 plotted against mother's average rank during her childbearing years ($R^2 = 0.36$, $P = 0.02$, $n = 14$, excluding GG; $R^2 = 0.02$, not significant, $n = 15$, including GG). Analysis includes all females whose rank was known during their childbearing years who were observed for at least 12 years as adults.

High rank may confer better access to food, both by enabling a female to acquire and maintain a core area of high quality and by affording her priority of access to food in overlap areas (*32*). Because of the mosaic distribution of vegetation at Gombe, some female core areas are likely to contain higher quality food than others. In addition, because core areas overlap almost completely, a high-ranking female may gain priority of access to preferred food sites in an overlap area. These modes of competition might explain why dominance behavior is less frequent in this species than in some others and why linear dominance hierarchies are hard to detect. If core areas are stable, competition is likely to be most intense when new or maturing females are attempting to establish their own core areas (*16*). This idea is consistent with the frequent observations of aggressive interactions from resident females to newly immigrant females at Gombe (*33*) and the Mahale mountains (*11*), the fact that PS and PM killed the infants of neighboring females as PM reached maturity (*31*, *34*), and observations of more frequent dominance interactions during the establishment of a new female group in captivity (*16*). In addition, clear dominance relationships may only be established between females whose core areas overlap, thus explaining the general lack of clearly defined linear dominance hierarchies. The stability of core areas, the acquisition of core areas, and the relationships of females whose core areas overlap are the focus of current research at Gombe.

More research is needed to understand how female chimpanzees achieve high rank. Some females, such as GG, acquired high rank by their own aggressive behavior. Other females, such as PM, have gained high rank through their mother's support. The fact that alliances with kin are sometimes important makes it all the more striking that young female chimpanzees often disperse to other communities. This underscores the suggestion that they are "forced" to do so in order to avoid inbreeding because their male relatives do not disperse, perhaps because of even stronger advantages to males from cooperation with relatives (*35*, *36*). More information on the relative importance of alliances in the acquisition of female dominance rank and the influence of dominance on reproductive success in other populations of chimpanzees might clarify why only half the females at Gombe disperse, whereas almost 100% do so in other populations (*9*). Finally, if the considerable degree of reproductive skew observed in the Gombe chimpanzees also occurs in other populations, this has implications for the future genetic diversity of this endangered species. As populations become small and isolated, there is a greater chance for the genetic diversity of the population to be reduced by the successful reproduction of a few dominant individuals (*37*).

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21. Our unpublished data suggest that time spent alone is even higher, and 80% core area size smaller, than published figures.
22. Of 14 females of known birth date born in the habituated community who experienced their first full anogenital swelling during the study, 6 settled as adults in the community, 5 transferred to other communities, and 3 disappeared. At the end of 1995, 5 of the 11 resident adult females in the community were born there, and 6 were immigrants.
23. Each year 11 to 18 adult females were observed in the community, of which a median of 16 (range 11 to 17) were observed for more than 10 days. Of these, a median of 87.5% (range 38.5 to 100%) could be assigned ranks in each 2-year block.
24. High-ranking females either gave no pant-grunts to any females or gave occasional pant-grunts to other high-ranking females, and received pant-grunts from middle- and low-ranking females. Middle-ranking fe-

males gave pant-grunts to high-ranking and some middle-ranking females and received them from low-ranking females and some other middle-ranking females. Low-ranking females rarely, if ever, received pant-grunts from any adult females but often gave them to middle- and high-ranking females. Fourteen females were assigned ranks of 1.5 or 2.5 during transitional periods.
25. Individuals are weighed by luring them up a rope attached to a spring balance by placing a piece of banana in a tin attached to the top of the rope. We fitted the natural logarithm of all weights of nonpregnant females to a curve using LOWESS. For females carrying infants, we subtracted the weight of an average infant of that age. This method makes no assumptions about the underlying shape of the curve, so the curve reflects the patterns in the data as accurately as possible. This standardizes weights by age, so we can find the residuals for a specific individual and determine whether she is bigger or smaller than average. We then regressed the average residual for each individual against dominance and found no significant relation. Thus, high-ranking individuals are not larger or smaller than lower ranking chimps.
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27. Eight females observed for at least two periods showed an increase in rank with age, one female showed a decrease, and 14 females showed no change ($P = 0.04$, $n = 9$, Sign test).
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29. Differences in survival are not statistically significant, but there is a suggestive trend with a Breslow-Gehan test ($P = 0.07$; $X^2 = 3.22$) when females who reach age 20 are placed into two ranks at age 21, high ($n = 9$) and low ($n = 6$). This test compensates for the reduction in sample size at later ages by weighting earlier deaths more heavily. However, statistical power of the test remains low because $n = 15$.
30. There were 11 deceased females, including the sterile female, GG, who were observed from or before the estimated age of 14 years (approximate age at first birth), whose rank and lifetime reproductive success could be determined. The relation between number of offspring surviving to 5 years and average rank is not significant when GG is included ($R^2 = 0.10$, $P = 0.34$, $n = 11$) but is significant when she is excluded ($R^2 = 0.60$, $P < 0.01$, $n = 10$).
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32. One food source that needs to be taken into account at Gombe is the provisioned bananas, although they comprised only a small proportion of the diet. There was considerable variation in the number of days on which individual females received bananas per year (median = 17, range 0 to 107). However, there was no significant relation between the number of days on which individual females received bananas between 1970 and 1990 and their dominance rank ($P = 0.62$, $n = 281$ in a multiple regression taking individual into account; $R^2 = 0.02$, $P = 0.5$, $n = 12$ when average number of banana days was regressed against average rank for each individual).
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36. _____ and C. Packer, in (*1*), p. 250.
37. The highest ranking female currently in the central community, FF, is 38 years old and has seven offspring, five over the age of 5 years. Two of her sons currently hold the alpha and beta position in the community, and one of her daughters resides in an adjacent community. She is the daughter of a high-ranking female (FLO) who produced at least 3 adult offspring, one of which was alpha male for 8 years. This family's genes are likely to gain significant representation in the total population in Gombe of less than 150 individuals.
38. G. A. Fox, in *Design and Analysis of Experiments*, S. M. Scheiner and J. Gurevitch, Eds. (Chapman & Hall, New York, 1993), p. 253.
39. We thank the government of Tanzania, Tanzania National Parks, and Serengeti Wildlife Research Institute for permission; A. Collins for ensuring the continuity of long-term data collection; numerous field assistants, students, and volunteers for data collection and analysis; T. Susman for organizing the demography database; and M. Tatar for assistance in performing the survival analyses. Many organizations have funded the project over the years, including the National Geographic Society, W. T. Grant Foundation, Wenner Gren Foundation, L. S. B. Leakey Foundation, Jane Goodall Institute, Carnegie Corporation, and NSF.

17 March 1997; accepted 10 June 1997

# Bypass of Senescence After Disruption of p21$^{CIP1/WAF1}$ Gene in Normal Diploid Human Fibroblasts

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Most somatic cells die after a finite number of cell divisions, a phenomenon described as senescence. The p21$^{CIP1/WAF1}$ gene encodes an inhibitor of cyclin-dependent kinases. Inactivation of p21 by two sequential rounds of targeted homologous recombination was sufficient to bypass senescence in normal diploid human fibroblasts. At the checkpoint between the prereplicative phase of growth and the phase of chromosome replication, cells lacking p21 failed to arrest the cell cycle in response to DNA damage, but their apoptotic response and genomic stability were unaltered. These results establish the feasibility of using gene targeting for genetic studies of normal human cells.

The replicative life-span of somatic cells reflects the number of cell divisions, not chronological time, and may contribute to organismic aging (*1*). Shortening of telomeres may be the molecular mechanism that triggers an irreversible arrest, referred to as senescence, of the prereplicative phase of growth in the cell cycle ($G_1$) (*2*). Genes that have been implicated in regulating senescence include tumor suppressors p53 (*3*) and RB1 (*4*), cyclin-dependent kinase (Cdk) inhibitors p21$^{CIP1/WAF1}$ (*5*) and p16$^{INK4a}$ (*6*), and several currently unidentified genes (*7*). Viral oncoproteins that interfere with p53 and RB1 cause bypass of senescence and extended life-span, followed by a decline designated as crisis (*8*). Two limitations have hampered studies of human senescence. First, viral oncoproteins may not completely inactivate their targets. Second, studies in rodents cannot be extrapolated to humans because of interspecies differences in the mechanisms of senescence and immortalization (*9*).

Introduction of null mutations into a cellular gene is a direct and unambiguous way to test the function of that gene. We were concerned that normal human diploid fibroblasts (HDFs) would senesce before two sequential rounds of gene targeting could be completed. Therefore, we developed strategies for efficient gene targeting in somatic cells (*10*), established a culture system that allows high single-cell cloning efficiency (*11*), and generated a new cell strain (LF1) (Fig. 1A) (*12*).

LF1 cells ($5 \times 10^7$ cells) at passage 7 were electroporated with a targeting vector containing a neomycin (*neo*)-resistance gene (Fig. 2A) (*13*). Twenty colonies were obtained and expanded into clonal cell strains. Southern (DNA) blotting analysis showed that three clones (HE1.2-1, HE1.3-2, HE3.2-1) (*14*) contained one targeted p21 gene copy (Fig. 2B). HE1.3-2 cells ($5 \times 10^7$ cells) were electroporated with a vector containing a hygromycin (*hyg*)-resistance gene (Fig. 2A), yielding 24 clones, one of which (HO7.2-1) had targeted the second p21 gene copy (Fig. 2B), and two of which had retargeted the *neo*-targeted gene copy (*15*, *16*). Protein immunoblotting analysis confirmed that the HO7.2-1 clone did not express p21 protein (Fig. 2C).

The hygromycin-resistant colonies were expanded into cell strains and passaged until senescence (Fig. 1C) (*17*). The 21 nontargeted (p21 +/−) strains senesced between passages 2 and 10 (mean passage 6.76 ± 2.55 SD) (*18*), whereas the HO7.2-1 strain did not cease proliferation until passage 19, when it displayed signs of crisis (*19*). During the period of extended life-span, no cell death was evident in HO7.2-1 cultures. Because in our experimental regimen one passage is equivalent to a minimum of two population doublings (PD) (*11*), loss of p21 resulted in quantitatively the same life-span extension (20

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